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Optimized QIAGEN DNeasy Blood & Tissue kit Protocol for Environmental DNA Extraction

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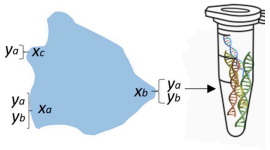

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We use this protocol and it's working

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Abstract

This document aims at providing a transparent method and detailing mandatory steps to produce reproducible 1) preparation of an eDNA filter, and 2) environmental DNA extraction.

Guidelines

The extraction of environmental DNA (eDNA) should be processed in the dedicated room of the ultraclean laboratory (i.e., with filtered air and positive air pressure) of the Maurice Lamontagne Institute, Fisheries and Oceans Canada. All samples, consumables, and material entering the room are cleaned with a 0.6% sodium hypochlorite solution. Laboratory users are trained to work in clean conditions (i.e., specific instructions about when to wear and change sterile gloves, coats, mobcaps, chirurgical masks, and overshoe protections) according to the SOP 020901_VXX. The extraction room is decontaminated between projects or every week (i.e., if a room is used for a project longer than a week) with a 0.6% sodium hypochlorite solution.

Materials

Equipment:

1. Tyvex lab coat (VWR #80200-600)
2. Disposable hair caps
3. Surgical mask
4. Scissors
5. Tweezers
6. Nacelles
7. Tube opener
8. -20 °C freezer
9. Pipettes 200 µL (Eppendorf P200)
10. Pipettes 1000 µL (Eppendorf P1000)
11. Racks
12. Vortexer
13. Thermomixer with 2 mL block adaptor (Eppendorf ThermoMixer Model C)
14. Microcentrifuge with rotor for 2 mL tubes (Eppendorf Model 5430)
15. Safety wash bottle of ethanol 96-100%
16. Safety wash bottle of Milli-Q water
17. Safety wash bottle of sodium hypochlorite 0.6 %
18. Solid trash
19. Liquid trash

Reagents:

1. Qiagen DNeasy Blood & Tissue kit (QIAGEN #69506)
2. EB Buffer (QIAGEN #19086)
3. AL Buffer (QIAGEN #19075)
4. ATL Buffer (QIAGEN #19076)
5. Proteinase K (QIAGEN #19133)
6. Ethanol 96-100%
7. Commercial sodium hypochlorite 6%

Consumables:

1. 2 mL microtubes (Ultident #87-B200-C)
2. 2 mL Eppendorf Safe-Lock microtubes (VWR #CA20901-505)
3. Lyse&Spin baskets with associated collection tubes (QIAGEN #19598)
4. Collection tubes (QIAGEN #19201)
5. Pipette tips with filter for P200 (VWR #CA89092-968) and P1000 (VWR #CA76416-026)
6. Kimwipes
7. Nitrile gloves
8. Filters: 1.2 or 1.5 µm GF, 47 or 25 mm (Millipore, Cat no. 1822-047, 1822-025, 1827-047 et 1827-025), and other possibilities.

Troubleshooting

Safety warnings

From the QIAGEN DNeasy Blood and Tissue Handbook (version 2022):

Safety Information (p. 7)

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view and print the SDS for each QIAGEN kit and kit component.
- DO NOT add bleach or acidic solutions directly to the sample preparation waste.
- Buffers AL and AW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach. If liquid containing these buffers is spilt, clean with a suitable laboratory detergent and water. If the spilt liquid contains potentially infectious agents, clean the affected area first with laboratory detergent and water, and then with 1% (v/v) sodium hypochlorite.

From the safety data sheet of the DNeasy Blood and Tissue Kit (50, revision date 25.08.2023):

- Buffer AL contains guanidine hydrochloride: harmful, irritant.

Hazard statements:
H302 + H332 Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.
H319 Causes serious eye irritation.

Precautionary statements:
Prevention:
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
Response:
P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention.

- Buffer AW1 contains guanidine hydrochloride: harmful, irritant.

Hazard statements:
H302 + H332 Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.

Precautionary statements:
Prevention:
P271 Use only outdoors or in a well-ventilated area.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
Disposal:
P501 Dispose of contents/ container to an approved waste disposal plant.

- Proteinase K contains proteinase K: sensitizer, irritant. Risk and safety phrases: *R36/37/38-42/43, S23-24-26-36/37*.

Hazard statements:
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Precautionary statements:
Prevention:
P261 Avoid breathing mist or vapours.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P284 Wear respiratory protection.
Response:
P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor (Québec: 1-800-463-5060).

From the safety data sheet of the Ethanol Solution 96% from ThermoFisher Scientific (version 24.12.2021):

- Ethanol solution 96%: flammable liquid, serious eye damage/irritation.

Hazard statements:
Highly flammable liquid and vapor
Causes serious eye irritation

Precautionary statements:
Prevention:
Use personal protective equipment as required
Wash face, hands and any exposed skin thoroughly after handling
Wear eye/face protection
Do not breathe dust/fume/gas/mist/vapors/spray
Use only outdoors or in a well-ventilated area
Keep away from heat/sparks/open flames/hot surfaces. - No smoking
Keep container tightly closed
Ground/bond container and receiving equipment
Use explosion-proof electrical/ventilating/lighting equipment
Use only non-sparking tools
Take precautionary measures against static discharge
eep cool

Response
IF exposed or concerned: Get medical attention/advice
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
In case of fire: Use CO2, dry chemical, or foam for extinction

From the safety data sheet of the sodium hypochlorite solution (10-15%) from ThermoFisher Scientific (version 13.10.2023):

- Sodium hypochlorite: corrosive to metals, respiratory irritation, skin burns and eye damage, toxic gas when in contact with acids.

Hazard statements:
May be corrosive to metals
Causes severe skin burns and eye damage
May cause respiratory irritation
Contact with acids liberates toxic gas

Precautionary statements:
Prevention
Take any precaution to avoid mixing with acids
Do not breathe dust/fumes/gas/mist/vapours/spray
Wear respiratory protection
Wash face, hands and any exposed skin thoroughly after handling
Keep only in original container
Use only outdoors or in a well-ventilated area
Wear protective gloves/protective clothing/eye protection/face protection

Response
IF INHALED: Remove person to fresh air and keep comfortable for breathing.
Immediately call a POISON CENTER/doctor (Québec: 1-800-463-5060)
IF SWALLOWED: Rinse mouth. Do NOT induce vomiting
IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/ shower
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
Wash contaminated clothing before reuse
Absorb spillage to prevent material damage

Before start

Buffer AL may form a precipitate upon storage. If necessary, warm to 56°C until the precipitate has fully dissolved.

Buffer AW1 and Buffer AW2 are supplied as concentrated solutions. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle to obtain a working solution.

Preheat a thermomixer at 56°C.

Filter Preparation

6m

1 Clean bench with water (i.e., Milli-Q water, hereafter water for cleaning procedures, to remove QIAGEN solutions with guanidine salts prior bleaching; see warnings for details), 0.6% sodium hypochlorite solution (i.e., to degrade DNA), and water (i.e. to rinse traces of sodium hypochlorite) before use.

2 Install all the material on the benchtop including 2 mL microtubes with Lyse&Spin baskets for extraction, pre-identified 2 mL Eppendorf Safe-Lock for back up, tweezers, scissors, nacelles, waste beaker, microtube opener, and gloves.

1m

3 Above a clean nacelle, take a filter with clean tweezers, unfold it, and cut it in half with clean scissors.
Note: Attemps to divide the filtrate equally on each half.

1m

4 Roll the filter half (filtrate inside) and put it in the extraction microtube (2 mL microtube with a Lyse&Spin basket) using clean tweezers. If DNA extraction will occur a subsequent day, put the filter half into a 2 mL microtube and store it at -20 °C. If DNA extraction will occur on the same day, keep it at room temperature on the bench.

30s

5 Roll the second half with tweezers, put it in a Eppendorf Safe-Lock 2 mL previously identified, and store it as a back up at -80 °C.

30s

6 Rinse tweezers, scissors, and nacelle with water, 0.6% hypochlorite bleach, water, and ethanol 96-100% (i.e., to dry equipment and avoid rust; hereafter, ethanol for cleaning procedures). Change gloves. Repeat steps 3 to 6 for each filter.

2m

7 Clean bench and material with water, 0.6% sodium hypochlorite solution, and water after use.

Critical Notes for DNA Extraction

8 *DO NOT TOUCH microtube edges with gloves while opening. Always use a microtube opener. In case of doubt for contamination, change gloves.*

DO NOT OPEN more than one microtube at a time to limit contamination.

ALWAYS do a quick spin before microtube opening to limit aerosols. In case of doubt for contamination, rinse the microtube opener with water, 0.6% sodium hypochlorite, and water.

ALWAYS change the pipette tip between microtubes when adding a solution, even if the same solution is added.

DNA Extraction

2h 24m 15s

9 Clean bench with water, 0.6% sodium hypochlorite solution, and water before use.

10 Clean pipettes and centrifuges by wiping them down with water, 0.6% sodium hypochlorite solution, water, and ethanol.

11 Install all the material on the benchtop including microtubes, reagents and collection tubes from the QIAGEN DNeasy Blood and Tissue Kit, and racks.

12 Prepare an extraction negative control for each extraction day.
Note 1: Use a filter of material and porosity identical to those from the project.
Note 2: If filters were frozen (i.e., usually moisten), then humidify the filter with Milli-Q water and proceed with steps 3 and 4. If filters were in silica beads then proceed with steps 3 and 4 without humidifying.

13 Change gloves and work under extractor hood or arm for all subsequent steps because of proteinase K (i.e., may cause breathing difficulties if inhaled).

14 Add 450 µL of ATL solution to each microtube.

15 Add 50 µL of proteinase K to each microtube.

16 Mix microtubes by inversion for few seconds. Make sure that the filter stays immerged in the solution.

17 Place microtubes in the thermomixer. Incubate for a minimum of 02:00:00 at 56 °C with shaking at 900 rpm for lysis of the filtrate.
Note: GF filters should not digest.

2h

18 Centrifuge microtubes at 18,000 g during 00:01:00 .
Note: If lysis solution is still present in the column after centrifugation, redo step 18 until all the solution went through the column. Make note in the excel sheet.

1m

19 Transfer the eluate to a new labeled 2 mL microtube.

Note: The microtube used with the Lysis&Spin basket was often leaking in the centrifuge at next steps which is why changing the microtube at this step is desirable.

20 Add 500 µL of AL buffer to each microtube. Change pipette tip between each microtube. Vortex.

21 Incubate in the thermomixer 00:10:00 at 56 °C . 10m

22 Quick spin.

23 Add 500 µL of ethanol 96-100% to each microtube. Change pipette tip between each microtube.

24 Mix microtubes by inversion for 00:00:15 . 15s

25 Quick spin.

26 Pipet up to 690 µL of the mix into a DNeasy column placed in a 2 mL collection tube.

27 Centrifuge 00:01:00 at 6,000 g. Discard flow-through and collection tube. 1m

28 Place the DNeasy column in a new collection tube.
Note: When collection tubes are discarded in the trash, be careful not to contaminate gloves and bench top.

29 Repeat steps 26 to 28 once all the solution went through the column.
Note: DNA is in the column.

30 Add 500 µL of AW1 buffer. Change pipette tip between each microtube.

31 Centrifuge 00:01:00 at 6,000 g. Discard flow-through and collection tube. 1m

32 Add 500 µL of AW2 buffer. Change pipette tip between each microtube.

33 Centrifuge 00:03:00 at 17,500 g. Discard flow-through and collection tube. 3m

34 Place the DNeasy column in a new collection tube and centrifuge again at 17,500 g for 00:02:00 to dry the column. 2m

35 Place the column into a 2 mL labeled Eppendorf Safe-Lock microtube.

36 Add 100 µL of EB buffer directly in the center of the membrane.
Note: We use EB buffer instead of the AE buffer provided in the Blood and Tissue kit as EB buffer is a TRIS buffer without EDTA. For rare species detection, we observed qPCR inhibition due to this small concentration of EDTA in the past.

37 Incubate at Room temperature for 00:05:00 . 5m

38 Centrifuge 00:01:00 at 6,000 g. 1m

39 Discard the column and store the 2 mL labeled Eppendorf Safe-Lock microtube with the eluate at 4 °C for eDNA detections within two weeks, at -20 °C for eDNA detections between 2 weeks and 3 months, and at -80 °C for eDNA detections later than 3 months.
Note: The storage protocol of DNA extracts is based on the duration prior the DNA detection step and the location of freezers at Maurice Lamontagne Institute. We favour the storage of DNA extracts at 4 °C within the ultraclean laboratory to limit contamination and to maximize chances of eDNA detections since freeze-thaw cycles limit detection of rare molecules (i.e., most likely due to deterioration of DNA's integrity). We favour the storage of DNA extracts within the ultraclean laboratory at -20 °C to limit contamination. We favour the long term storage of DNA extracts at -80 °C to maximize the preservation of DNA integrity, when no detections are planned.

40 Throw liquid bench top trash into the liquid trash of the laboratory.

41 Rinse the liquid bench top trash with water, discard this water in the liquid trash of the laboratory, then clean it with water, 0.6% sodium hypochlorite solution, and water.

42 Clean bench with water, 0.6% sodium hypochlorite solution, and water.

43 Rinse microtube racks by spraying them with 0.6% sodium hypochlorite solution, and rinsing them with water.

Protocol references

DNeasy Blood & Tissue Kits (qiagen.com)

Safety Data Sheets - QIAGEN

msds (fishersci.ca)

msds (fishersci.com)